A Semiempirical Computational Study of Electron Transfer Reactivity of One- vs. Two-Ring Model Systems for Anthracycline Pharmacophores. I. A Rationale for Mode of Action

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ABSTRACT

The redox capacities of 5-hydroxy-1,4-naphthaquinone (VI), 5-8-dihydroxy-1,4-naphthaquinone (VII), and 5,8-dihydroxy-1,4-naphthaquinone imine (VIII) as model systems for the pharmacophore of aclacinomycin A, adriamycin/daunomycin, and 5-iminodaunomycin (5IDN), respectively, along with 1,4-naphthaquinone (V), 1,4-benzoquinone (IV), and 1,4-benzoquinone imine (IX), have been investigated by the AM1 semiempirical method. The reduction activation of the parent (Q) model systems to their various redox states [quinone radical anion ($Q^{-\bullet}$), semiquninone ($QH^{\bullet}$), semiquinone anion (QH^{-}), and hydroquinone (QH_2)], the redox capacities of the redox states, and the intermolecular electron self-exchange processes between the redox states and electron transfer reactions from the redox states to molecular oxygen have been examined using reaction enthalpies, adiabatic ionization potentials and electron affinities, and absolute and adiabatic electronegativities. Keto–enol transformations and the effects of solvation and H bonding on keto–enol tautomers of VI and of the hydroquinones of VI and VIII have also been assessed. The results indicate that the reactivity of VIII, relative to that of VII, may not be diminished. VI, however, appears to be less reactive than VII, and this suggests clues for the reduced toxicities of aclacinomycin A. Overall, the results suggest that the experimentally observed reduced cardiotoxic effects of 5IDN may be explained by changes in electron configuration and/or electron density and in geometry, such as changes in planarity that accompany enol to keto transformations in the reduction byproduct of 5IDN (Bird et al.[7])—that is, between the hydroquinone (II) of naphthacenedione (I) and naphthacenone (III). Moreover, the results

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suggest that the two-electron reduction product, Q^{-2}, of the drugs can be the reductant that produces reactive dioxygen species, such as $O_2^{-\bullet}$ and $HO_2^{\bullet}$, via electron transfer to molecular oxygen as opposed to $QH^{\bullet}$ and QH_2, which have been postulated to be responsible for electron transfer. This possibly new role for Q^{-2} may be important in cardioxicity, particularly in aprotic and/or hydrophobic media.

Introduction

Anthracyclines are a class of tetracyclic antibiotics used in human anticancer chemotherapy, and they have been highly investigated for the purpose of understanding their modes of action.[1,2] Despite their dissimilarity in structures, they have all been proposed to produce strand breaks in DNA through oxidoreduction processes and reductive activation of molecular oxygen.[2] In addition to this antitumor activity, the redox-mediated chemistry of the two anthracyclines, adriamycin and daunomycin (Chart I) leads to the formation of reactive oxygen species[3] and is thought to be responsible for the serious side-effects of acute cardiotoxicity. Indeed, one of the primary reasons for the intense interest in the anthracyclines has been motivated by the desire to find a way to alleviate the cardiotoxic effects. Although much success has not been accomplished in this regard, a semisynthetic derivative of daunomycin (DN), 5-iminodaunomycin (5IDN) (Chart I), has been shown to have less cardiotoxicity than daunomycin while retaining significant antitumor activity.[4]

One rationale for the lower cardiotoxicity of 5IDN has been proposed to be its diminished capacity for catalytically producing reactive oxygen species[5]; and, in fact, 5IDN has been described as a redox-incapacitated anthracycline.[6] These conclusions stemmed from the electrochemical results of Lown et al.,[5] which indicated that an aqueous solution of 5IDN was more difficult to reduce than DN and that the reoxidation of 5,11-dihydro-5-iminodaunomycin (3) (Chart I) in aqueous solution was much more difficult than the reoxidation of the reduced DN. They tentatively had attributed the unusual stability of 3 to strong H bonding. The rationalization that 5IDN is incapable of catalyzing efficiently the reduction of molecular oxygen (because it is difficult to reduce 5IDN and/or to reoxidize the reduced 5IDN) should, however, be

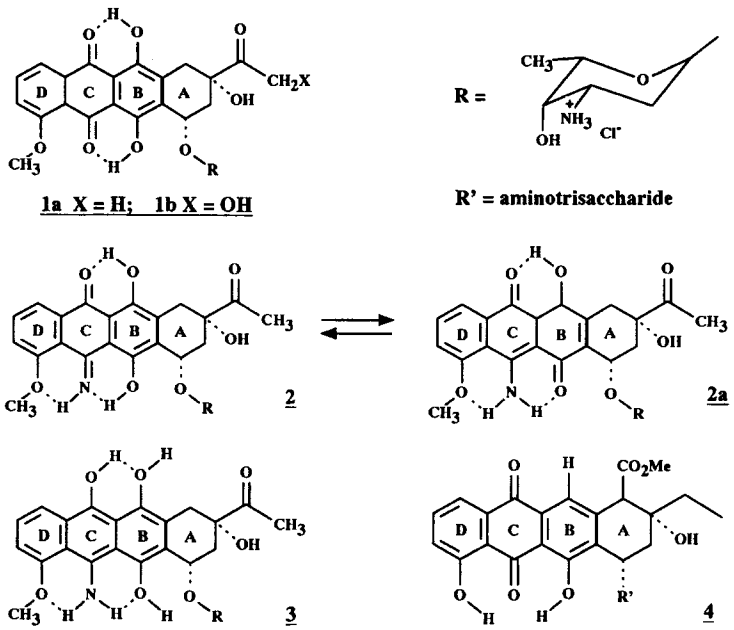


CHART I. Structural formulae of duanomycin (1a), adriamycin (1b), 5-iminodaunomycin (2), 5,11-dihydro-5-iminodaunomycin (3), aclacinomycin A (4).

open to question from the point of view of quantum chemical considerations. The issue of 5IDN being a redox-incapacitated anthracycline was, in fact, experimentally investigated by Bird et al.,[7] who proposed a possible explanation for the inefficiency of 5IDN to catalyze *in vivo* the reduction of molecular oxygen to be the facile formation of naphthacenedione (I) whose hydroquinone (II) tautomerizes to naphthacenone (III) (Scheme 1) in preference to reduction of molecular oxygen.[7] The possible mechanistic explanation offered by Bird et al.[7] suggests a rather interesting and important role for keto and enol forms of the drug metabolites. Although the tautomerization might not be considered a minor structural difference, it is not obvious that it can lead to a major difference in redox property and/or biochemical activity. This observation also raises the question as to what extent the chemical reactivity under consideration should be expected to be different, and what exactly might be responsible for this difference. In any case, the difference in reactivity arising from such a variation in structure, specially if it is indeed responsible for the reduced toxicity of 5-iminodaunomycin, has significant implications from the point of view of design of anthracyclines with reduced toxicity. Thus, further understanding of the inherent reactivity is important. In light of this, we have initiated a computational program to study the reactivities of model systems for the pharmacophores of the anthracycline family of drugs. The thrust of this effort is to gain some insight from molecular modeling to understand molecular-level features that govern chemical reactivity but may not be apparent from the molecular structure and raw experimental data.

It can be presumed that the various redox processes that the anthracyclines undergo originate from the presence of the quinone moiety in the pharmacophore. Since this quinone moiety in DN is replaced by a quinone imine in 5IDN (Chart I), the difference in chemical reactivity of the two cases is of interest. Consideration of the following general mechanistic scheme, which has been advanced to be compatible with membrane lipid peroxidation and/or DNA damage, should provide a proper perspective[2,8]:

$$Q + e^- + H^+ \rightarrow QH^\cdot \quad (1)$$

or

$$Q + 2e^- + 2H^+ \rightarrow QH_2 \quad (2)$$

$$QH_2 + O_2 \rightarrow QH^\cdot + HO_2^\cdot \quad (3)$$

or

$$QH^\cdot + O_2 \rightarrow Q + HO_2^\cdot \quad (4)$$

$$HO_2^\cdot \rightleftharpoons O_2^{\cdot -} + H^+ \quad (5)$$

The superoxide ion can, in subsequent steps, undergo the following further reactions:

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$H_2O_2 + O_2^{\cdot -} \rightarrow OH^- + OH^\cdot + O_2$$

(Haber-Weiss reaction)

$$OH^\cdot + DNA \rightarrow H_2O + DNA^\cdot$$

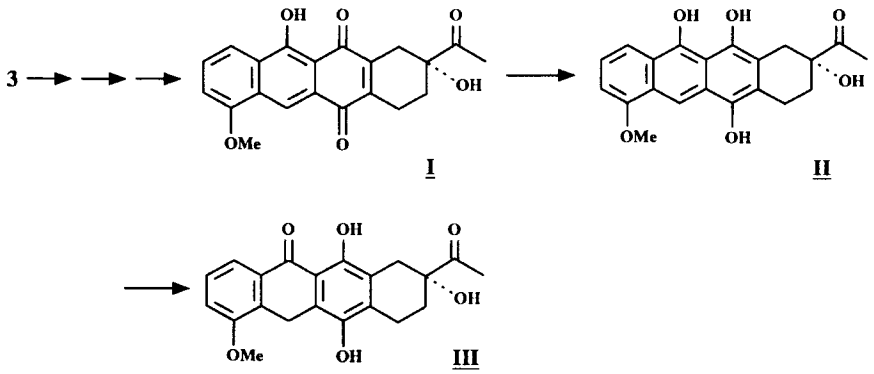
(deoxyribose or base attack)

$$DNA^\cdot + O_2 \rightarrow DNA - OO^\cdot \rightarrow$$

(strand scission or base decomposition/elimination)

It is generally believed that the dose-related cardiotoxicity of the anthracyclines leading to cardiac lipid peroxidation arises from the same reactive oxygen species.[2,8]

The reactions relevant to our study in the preceding scheme are the reductive activation of the anthracyclines, represented by Q (1 and 2) and the electron transfer reactions from $QH^\cdot$ (semiquinone) and QH_2 (hydroquinone) to molecular



SCHEME 1. Reduction of 5,11-dihydro-5-iminodaunomycin(3) to the hydroquinone of naphthacenedione(II).

$$Q \xrightarrow{1} Q^{\cdot -} \xrightarrow{2} QH^{\cdot} \xrightarrow{3} QH^{-} \xrightarrow{4} QH_2$$

SCHEME 2. A possible sequence of steps in the reductive activation of quinones.

oxygen (3 and 4). Moreover, one electron reduction of Q and $QH^{\cdot}$ leads to $Q^{\cdot -}$ and QH^{-}, respectively. In addition to electron transfer to O_2, there can also be competing electron exchange reactions between the different reductively activated Q's (i.e., Q, $Q^{\cdot -}$, $QH^{\cdot}$, QH^{-}, and QH_2).

The present study is thus aimed at getting further insight into the relative reactivities of two-ring model systems (Chart II) for the substituted naphthoquinone part (rings B and C in Chart I) of the pharmacophore of the anthracyclines, one of the rings being the quinone or quinone imine moiety. Benzoquinone (IV) and benzoquinone imine (IX) are also included for the purposes of comparison. Within the theoretical framework of the AM1 semiempirical method, we have examined the relative reactivities of V–VIII (Chart II) along with IV and IX by comparing the reaction enthalpies for electron and proton attachments steps in the reductive activation of V–VIII to their various redox states (Q, $Q^{\cdot -}$, $QH^{\cdot}$, QH^{-}, and QH_2). Reaction enthalpies for intermolecular electron self-exchange processes between the redox states, and reaction enthalpies for electron transfer from the various redox states to singlet and ground state O_2, are also assessed. The relative redox capacities of the model systems were also assessed using AM1-calculated adiabatic ionization potentials (IP_{ad}) and electron affinities (EA_{ad}) and electronegativity. Keto-enol transformations and the effects of solvation and hydrogen bonding were also examined in VI and its hydroquinone since this hydroquinone is part of II (Scheme 1) and the reduced form (QH_2) of aclacinomycin A (rings B and C, Chart I and Scheme 2). The results of the computational investigations seem to suggest strongly that the reduced cardiotoxic effects of 5IDN may be explained by changes that accompany enol to keto transformations (Scheme 1); more specifically, they may be due to the resultant changes in electron configuration and/or electron density and in geometry, such as changes in planarity.

Computational Approaches

AM1-derived parameters, such as heats of formation, ionization potentials, orbital energies (HOMO, SOMO and LUMO), and protonation energies, have been successfully used to investigate reactivity of biologically important molecules,[9–12] single electron transfer reactions (including radically mediated oxidations and reductions), and hypolipidemic activity of phthalmide and related compounds.[13] In particular, the work by Brewster et al.[12] has shown a linear correlation between AM1-derived adiabatic ionization potentials and the log of ferricyanide-mediated oxidation of substituted 1, 4-dihydropyridines. They further demonstrated that useful relationships could be generated between oxidation rates and absolute electronegativity. The absolute electronegativity (X_V), defined as the average energy of the ionization potential (IP) and the electron affinity (EA), and the absolute hardness (N_V), defined as half the difference between IP and EA, are considered to be useful parameters for the study of electron transfer reactions.[12] Moreover, the incorporation of these parameters into molecular orbital theory[14] based on the tenets of Koopmans' theorem has led to the definition of X_V as the negative of the average HOMO and LUMO energies and the defi-

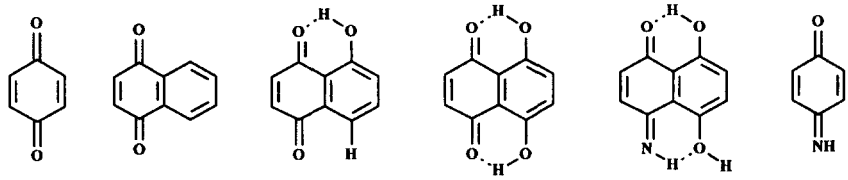


CHART II. Structural formulae for some model systems for the pharmacophore of the anthracyclines. IV = 1,4-benzoquinone; V = 1,4-naphthaquinone; VI = 5-hydroxy-1,4-naphthaquinone (juglone); VII = 5,8-dihydroxy-1,4-naphthaquinone; VIII = 5,8-dihydroxy-1,4-naphthaquinone imine; IX = 1,4-benzoquinone imine. All are part of the anthracycline pharmacophore; specifically: VI is part of aclacinomycin A, VII is part of adriamycin and daunomycin, and VIII is part of 5-iminodaunomycin.

nition of N_V as half the HOMO–LUMO difference.[15,16] X's determined this way from semiempirical HOMO/LUMO energies were suggested to be useful quantities in describing chemical reactivity.[12]

Since the primary motivation in this work is to assess the relative reactivities of the model systems described earlier and to get further insight into the origins of the reactivities, it was thus anticipated that it would be reasonable and adequate to use the computationally fast, but less accurate, AM1 semiempirical method for assessing trends in the chemical reactivities of the model systems. Moreover, AM1-calculated heats of formation (H_f's) are generally expected to be acceptable (i.e., close to experimental values). Nevertheless, since the species to be considered include not only neutral and charged closed-shell systems but also neutral and charged radicals and species, results of both UHF (unrestricted Hartree-Fock) and ROHF (restricted open-shell Hartree-Fock) calculations for radicals will be presented. If relaxation and correlation effects are insignificant, the UHF and ROHF results should give very similar heats of formation. In most cases, the data will be presented as two sets: RHF/UHF, where RHF (restricted Hartree-Fock) was used for closed-shell systems and UHF for radicals, and RHF/ROHF, where ROHF was used for radicals. It will be shown that, in many instances, the same conclusions can be reached from the two sets of calculations. It will also be shown that the magnitudes of the effects of the UHF calculations may be very different for $Q^{\bullet -}$, $QH^{\bullet}$, and $QH_2^{+\bullet}$, although they may be very similar for a given type of redox state (e.g., $QH^{\bullet}$ arising from different parent molecules). Thus, while the RHF/ROHF and RHF/UHF results may lead to the same trend in reactivity, it will be shown that the magnitudes of the reaction enthalpies can actually be considerably different.

Methods

The quinone (Q) moieties of the pharmacophores of the drugs can be reductively activated to hydroquinone (QH_2) incorporating two electrons and two protons: $Q + 2e + 2H^+ \rightarrow QH_2$. A possible sequence of steps for these reactions can be as follows:

$$Q \xrightarrow{1} Q^{\bullet -} \xrightarrow{2} QH^{\bullet} \xrightarrow{3} QH^- \xrightarrow{4} QH_2$$

where steps 1 and 3 are electron attachment steps, steps 2 and 4 are proton attachment steps, and Q, $Q^{\bullet -}$, $QH^{\bullet}$, QH^-, and QH_2 represent the various redox states (quinone, quinone radical anion, semiquinone, semiquinone anion, and hydroquinone, respectively).

Structures, energies, and energetic properties of the various model systems in Chart II, along with some selected keto and enol forms, were calculated utilizing RHF and UHF AM1 formalism[17] (both implemented in MOPAC[18] [version 5] for closed- and open-shell systems, respectively. The MOPAC program used was part of an Insight II package from BIOSYM.[19] Open-shell systems were also calculated by the ROHF method primarily using AMPAC (Version 4.5, Semichem).[20] All input structures were minimized using PCMODEL[21] prior to MOPAC or AMPAC calculations. Molecular geometries and energies obtained from MOPAC and AMPAC involved Broyden-Fletcher-Goldfab-Shanno optimization with respect to all structural variables. The PRECISE (in all cases) and NLLSQ (in most cases) options were also used. In some cases, the minimization required several thousand cycles with no significant change in the energy and the gradient (apparently because the minimum is a shallow potential energy well), and the minimization had to be terminated once the gradient was about or less that 1 kcal/mol/Å.

Gas phase reaction enthalpy calculations for electron attachment were accomplished by determining ΔH of the reaction $Y + e^- \rightarrow Y^-$—that is, $\Delta H = H_f(Y^-) - H_f(Y)$, where Y is Q or $QH^{\bullet}$ and H_f is the calculated heat of formation. Reaction enthalpies for proton attachment were calculated by determining ΔH of the reaction $Y + H^+ \rightarrow YH$ that is—$\Delta H = H_f(YH) - H_f(Y)$, where Y is $Q^{\bullet -}$ or QH^-. Within the validity of Koopmans' theorem,[22] orbital energies were used to calculate absolute electronegativities (X_V) and absolute hardness (N_V). Thus, X_V was estimated as the negative of the average HOMO and LUMO energies for closed-shell systems and the negative of the average SOMO (alpha-orbital) and LUMO (lowest empty beta-orbital) energies for open-shell systems; and N_V was determined as half the HOMO–LUMO difference for closed-systems and half the SOMO–LUMO (beta-orbital) difference for open-shell cases.

Adiabatic ionization potentials (IP_{ad}) were calculated as the enthalpy of the reaction[23] $X \rightarrow X^* + e$, where X = Q, $Q^{\bullet -}$, $QH^{\bullet}$, QH^-, and QH_2, whereas adiabatic electron affinities (EA_{ad}) were obtained as the negative of the enthalpy of the

reaction[23] $X + e \rightarrow X^-$ where $X = Q, Q^{-\bullet}, QH^{\bullet}, QH^-$, and QH_2. ROHF-determined heats of formation were used for all radicals whose IP_{ad}'s and EA_{ad}'s were determined. IP_{ad}'s and EA_{ad}'s were, in turn, used to calculate adiabatic electronegativities (X_{ad}) defined as half the sum of IP_{ad} and EA_{ad}.

Solvation free energies were calculated by the AM1-SM2 1SCF option (AMPAC 4.5), which permits only electronic relaxation.[24]

Results

ENERGETIC PROPERTIES

Various AM1-calculated energetic properties (heats of formation, orbital energies [HOMO, SOMO and LUMO], RHF/UHF- and RHF/ROHF-calculated reaction enthalpies for electron and proton attachment steps, absolute and adiabatic electronegativity) and expectation values (S^2 for radicals) of IV–IX are presented in Tables I through III. Although not presented as such in Table I, the negative of the HOMO, SOMO, and LUMO orbital energies can be used to obtain vertical ionization potentials (IP_V) and electron affinities (EA_V) in accordance with the tenets of Koopmans' theorem.[22] Koopmans' IPs, to a first approximation, are expected to be reasonably comparable to experimental IPs, while Koopmans' EAs are generally unreliable.[25] In a related work on IV, IX, and benzoquinone diimine, we have determined that absolute electronegativities calculated from Koopmans' IPs and EAs correlate with adiabatic electronegativities (calculated from IP_{ad}'s and EA_{ad}'s) satisfactorily.[26,†] This is probably so because the Koopmans' IPs and EAs we calculated were overestimated and underestimated, respectively, compared to the corresponding adiabatic ones, thus leading to a cancellation of errors. As shown from the plot of X_V vs. X_{ad} in Figure 1, the good agreement between absolute electronegativities (Koopmans'), X_V (Table I), and adiabatic electronegativities, X_{ad} (Table II) would permit one to use either parameter to compare the various redox states of V–VIII. As can be gleaned from the expectation value ($\langle S^2 \rangle$) data in Table II, there is considerable spin contamination in $Q^{-\bullet}$ and $QH_2^{+\bullet}$, and even more so for $QH^{\bullet}$. Interestingly, $QH^{\bullet}$'s from the different parent molecules have very similar % spin contamination and/or expectation values. However, the same cannot be said for $Q^{-\bullet}$ and $QH_2^{+\bullet}$. The effect of spin contamination appears to manifest as a lowering in H_f's as can be seen from the comparison of UHF- and ROHF-calculated H_f's in Table III. From the data presented in Table II and Figure 1, there is a good correlation between the % spin contamination and the difference in H_f's calculated by the UHF and ROHF methods ($\Delta\Delta H_f$). The good correlation observed can be interpreted as an indication of internal consistency of the data and may suggest that the UHF results may be used to assess the relative reactivity of the model systems in a qualitative sense. However, if reaction enthalpies are to be used to determine relative reactivity, UHF results alone may not be sufficient and additional evidence should be sought using ROHF results for radicals. Since the relative reactivities to be examined in this work rely considerably on reaction enthalpies, both the RHF/UHF and RHF/ROHF results are presented so that one can assess where they agree or differ. This discussion will, however, focus on the RHF/ROHF results, and only some of the cases in which the RHF/UHF results lead to a different conclusion will be pointed out. Also, comparison of relative reactivity will focus on V–VIII since work on IV and IX will be reported elsewhere and they are included here only for completeness. Moreover, data on VIIIc are used for the most part for the comparison since VIIIc is the most stable form of VIII (Scheme 3 and Table I) and consideration of VIIIa and VIIIb will not change the conclusion of the results.

Brewster et al.[12] have used the expression $\Delta E = (X_{D^0} - X_{C^0})^2/4(N_D + N_C)$ to estimate the resulting change in energy when an electron is transferred from C to D in an oxidation. Thus, the difference in electronegativity is expected to drive

[†] We have investigated vertical and adiabatic ionization potentials and electron affinities of the five redox states of 1,4-benzoquinone (IV), benzoquinone imine (IX), and benzoquinone diimine in detail. A good linear correlation (with correlation coefficient better than 0.95 and a slope close to 1) between vertical (RHF/UHF- and RHF/ROHF-determined) IPs and (RHF/UHF- determined) EAs (of the various states taken together) and their adiabatic (RHF/UHF- and RHF/ROHF-determined) IPs and EAs, respectively, was observed. Except for the fact that RHF/UHF IPs are overestimated and RHF/UHF EAs are underestimated, the analysis demonstrated that the relative values for a given redox state from different parent molecules should be fairly accurate, thus allowing qualititative to semiqualititative comparison of the various redox states. Comparison of RHF/UHF adiabatic IPs and EAs with the corresponding RHF/ROHF ones showed that the RHF/UHF adiabatic IPs are shifted by a constant of 1.2 kcal/mol and the RHF/UHF adiabatic EAs by −0.88 kcal/mol. This observation suggests that RHF/UHF adiabatic IPs and EAs can be used for comparative studies.

TABLE I.
AM1-Calculated Heats of Formation (H_f), Orbital Energies (HOMO, SOMO, LUMO), Absolute Electronegativities (X_V), and Absolute Hardness (N_V).[a]

	Redox state	H_f	HOMO[b]	LUMO	X_V	N_V
IV	Q	−25.1	−10.876	−1.735	6.305	4.571
	$Q^{-\bullet}$	−76.9	(−2.771)	(3.891)	−0.56	3.331
	$QH^{\bullet}$	−41.5	(−9.137)	(−1.288)	5.212	3.925
	QH^{-}	−86.6	−2.844	5.391	−1.275	4.118
	QH_2	−65.7	−8.725	0.219	4.253	4.472
V	Q	−15.9	−10.257	−1.547	5.902	4.355
	$Q^{-\bullet}$	−65	(−2.755)	(3.445)	−0.345	3.1
	$QH^{\bullet}$	−29.2	(−8.74)	(−1.205)	4.973	3.768
	QH^{-}	−72.5	−2.801	4.226	−0.713	3.514
	QH_2	−43.8	−8.24	−0.491	4.366	3.875
VI	Q	−61.4	−9.621	−1.675	5.648	3.973
	$Q^{-\bullet}$	−114.6	(−2.969)	(3.258)	−0.145	3.114
	$QH^{\bullet}$	−74.6	(−8.865)	(−1.463)	5.164	3.701
	QH^{-}	−123.7	−3.069	4.036	−0.484	3.553
	QH_2	−87.9	−8.154	−0.431	4.293	3.862
VII	Q	−105.8	−9.201	−1.788	5.495	3.707
	$Q^{-\bullet}$	−162.1	(−3.132)	(3.003)	0.129	3.068
	$QH^{\bullet}$	−117.4	(−8.595)	(−1.507)	5.051	3.544
	QH^{-}	−167.1	−3.123	4.018	−0.448	3.571
	QH_2	−129.7	−7.903	−0.424	4.164	3.740
VIII, a	Q	−53.1	−9.113	−1.353	5.233	3.88
	$Q^{-\bullet}$	−102.3	(−2.799)	(3.736)	−0.469	3.268
	$QH^{\bullet}$	−75.4	(−7.951)	(−1.071)	4.511	3.44
	QH^{-}	−113.8	−2.585	4.162	−0.789	3.374
	QH_2	−85.2	−7.463	−0.12	3.792	3.672
B[c]	$QH^{\bullet}$	−66.4	(−8.439)	(−0.586)	4.513	3.927
	QH^{-}	−99	−2.545	4.204	−0.83	3.375
VIII, b	Q	−52.5	−8.871	−1.5	5.186	3.686
	$Q^{-\bullet}$	−105.4	(−2.991)	(3.492)	−0.251	3.242
	$QH^{\bullet}$	−64.5	(−8.195)	(−1.202)	4.699	3.497
	QH^{-}	−106.1	−2.761	4.141	−0.69	3.451
	QH_2	−79.7	−7.888	−0.446	4.167	3.721
B[c]	$QH^{\bullet}$	−65.0	(−8.408)	(−0.872)	4.64	3.768
	QH^{-}	−104.7	−2.768	4.159	−0.696	3.464
VIII, c	Q	−55	−8.277	−1.72	4.999	3.279
	$Q^{-\bullet}$	−111.3	(−3.108)	(2.998)	0.055	3.053
	$QH^{\bullet}$	−75.4	(−7.952)	(−1.072)	4.512	3.44
	QH^{-}	−113.8	−2.585	4.103	−0.789	3.374
	QH_2	−85.2	−7.461	−0.117	3.789	3.672
B[c]	$QH^{\bullet}$	−74.5	(−8.305)	(−1.103)	4.704	3.601
	QH^{-}	−115.7	−2.794	4.262	−0.734	3.528

[a] H_f is in kcal / mol; all other parameters are in eV.
[b] Values in parentheses are SOMO alpha-orbital energies with their LUMO counterparts being for empty beta orbitals.
[c] Refers to route B in Scheme 3.

the reaction while the sum of the hardness values has the opposite effect. Consideration of the absolute hardness data did not, however, seem to provide a single order for all systems. In a related article,[26] we had compared N_V and N_D data in a correlation plot and there was considerable scatter with a correlation coefficient (R^2) < 0.6, although a better fit was found (with R^2 about 0.86) when the data for $QH^{\bullet}$'s were left out. Since the $QH^{\bullet}$'s are postulated to play important roles in the mode of action of the drugs, comparison of reactivity of redox states with the exclusion of $QH^{\bullet}$'s was not thought useful and the hardness data are not considered further.

REDUCTIVE ACTIVATION

Reaction enthalpies for the reductive activation involving electron and proton attachment steps of Scheme 2 are provided in Table IIIa, Section I. It is apparent from the data that the RHF/ROHF results are higher (less favorable) than the corresponding RHF/UHF results for steps 1 and 2. The RHF/ROHF results are, however, lower (more favorable) for step 3. The RHF/ROHF results indicate that, of the two electron attachment steps (1 and 3), step 3 is more favorable. This is, thus, one case in which the RHF/UHF results can lead to misleading conclusions. It is of interest to note that both the RHF/UHF and RHF/ROHF methods give the same reaction enthalpies for the overall conversion of $Q \rightarrow QH_2$.

The electron and proton attachment reactions of Scheme 2 (steps 1–4) are listed as reactions 1–4, respectively, in Table IV. The RHF/ROHF-calculated reaction enthalpies of steps 1–4 (Scheme 2)—that is, reactions 1–4 (Table IVa)—are compared with other electron and proton attachment

TABLE II.
Some AM1-Calculated Parameters for Various Redox States of V, VI, VII, and VIIIc.

	X_{ad}	X_V	$\langle S^2 \rangle$	% spinct	$\Delta\Delta H_f$	
V	Q	134.2	136.1			
	$Q^{-\bullet}$	−9.7	−8	0.83	9.1	3.8
	$QH^{\bullet}$	113.0	114.7	1.35	79.7	11.5
	QH^-	−17.2	−16.4			
	QH_2	99	100.7			
	$QH_2^{+\bullet}$			0.82	8.53	4.0
VI	Q	128.5	130.2			
	$Q^{-\bullet}$	−4.8	−3.3	0.98	30.9	4.3
	$QH^{\bullet}$	117.3	119.1	1.35	80.3	11.5
	QH^-	−10.3	−11.2			
	QH_2	97.8	99			
	$QH_2^{+\bullet}$			0.93	24	5.1
VII	Q	124.9	126.9			
	$Q^{-\bullet}$	0.1	3	1.03	37.8	4.5
	$QH^{\bullet}$	115.2	116.5	1.39	85.8	12.2
	QH^-	−9.6	−10.3			
	QH_2	94.9	96.0			
	$QH_2^{+\bullet}$			0.85	13.3	4.3
VIIIc	Q	113.6	115.3			
	$Q^{-\bullet}$	2.0	1.3	1.06	41.5	6.5
	$QH^{\bullet}$	101.2 (106.9)	104.0 (108.5)	1.37 (1.44)	82.9 (92)	10.2 (12.4)
	QH^-	−17.1 (−15.6)	−18.2 (−16.9)			
	QH_2	85.9	87.4			
	$QH_2^{+\bullet}$			1.06	40.9	6.2

X's and $\Delta\Delta H_f$'s are in kcal/mol; spinct = spin contamination; values in parentheses for VIIIc are for route B (Scheme 3); $\Delta\Delta H_f = H_f(\text{ROHF}) - H_f(\text{UHF})$.

reactions (reactions 5, 8, and 9, Table IVa) and one-electron oxidation of $QH^{\cdot}$ and QH_2 in Table IVb. Using the RHF/ROHF reaction enthalpies of Table IVb, the following qualitative to semiquantitative decreasing order in relative reactivity can be obtained:

Reaction	Relative Reactivity
1.	VII > VIIIc > VI > V
2.	VIIIc > V > VI > VII
3.	VII > VI > V > VIIIc
4.	VIIIc > V > VI > VII
5.	V > VI > VII > VIIIc
6.	VIIIc > VII > V > VI
7.	VIIIc > VII > VI > V
8.	VIIIc > VII > VI > V
9.	VIIIc > VII > VI > V

Semiquinone formation

$Q \rightarrow QH^{\cdot}$: VIII > V > VI > VII

Hydroquinone formation

$Q \rightarrow QH_2$: VIIIc > V > VI > VII

This analysis strongly suggests that, except for electron attachment reactions 1 and 3 and reaction 5, the reactions considered in Table IVa should be thermodynamically more favorable or as favorable in VIIIc compared to VII. The calculated gas phase reaction enthalpy data taken as a whole do not suggest that VIIIc is less reactive toward the reductive activation process proposed in the mechanism of action of the drugs [vide supra, Eqs. (1) and (2), Introduction]. The same conclusion holds even when different forms of VIII are considered. Although there will certainly be some errors in the calculated H_f's (hence the comparison can only be qualitative to semiquantitative at best), the trends observed should be reasonably accurate.

REDOX CAPACITY

The redox capacity of the reductively activated forms of V–VIII as reducing and oxidizing agents can be assessed by evaluating the ionization potentials and electron affinities, respectively, of the various species. The AM1 RHF/ROHF calculated adiabatic ionization potentials (IP_{ad}) and electron affinities (EA_{ad}) given in Table IVc can be used to obtain the following qualitative to semiquantita-

TABLE IIIa.
Reaction Enthalpies (kcal / mol) for the Electron and Proton Attachment Steps in Scheme 1 and for the Overall Conversion of Q → QH_2.

		Steps				
		1	2	3	4	Overall
I.	RHF / UHF[a]	Results				
	IV	−51.8	35.4	−45.1	20.9	−40.6
	V	−49.1	35.9	−43.3	28.7	−27.8
	VI	−53.2	40	−49.1	35.8	−26.5
	VII	−56.2	44.7	−49.7	37.4	−23.8
	VIII, a	−49.3	26.9	−38.4	28.7	−32.1
		−49.3	35.9	−32.6	13.9	−32.1
	VIII, b	−52.9	40.9	−41.6	26.4	−27.2
		−52.9	39.5	−38.8	24.9	−27.3
	VIII, c	−56.3	35.8	−38.4	28.7	−30.2
		−56.3	36.8	−41.2	30.5	−30.2
	IX	−46.5	22.3	−40.9	18.6	−46.5
II.	RHF / ROHF[a]	Results				
	IV	−48.7	41.6	−54.4	20.9	−40.6
	V	−45.3	43.5	−54.8	28.7	−27.9
	VI	−48.9	47.2	−60.6	35.8	−26.5
	VII	−52.1	52.7	−61.9	37.4	−23.9
	VIIIc	−49.8	39.6	−48.6	28.7	−30.1
		−49.8	42.7	−53.6	30.5	−30.2
	IX	−40.7	24.7	−49.1	18.6	−46.5

[a] UHF and ROHF methods were used in I and II, respectively, for radicals.

TABLE IIIb.
Comparison of UHF- and ROHF-Calculated H_f's for Various Radicals (kcal / mol).

		IV	V	VI	VII	VIII, c	IX
$Q^{-\cdot}$	UHF	−76.9	−65	−114.6	−162.1	−111.3	−22.9
	ROHF	−73.8	−61.2	−110.3	−157.8	−104.8	−17.1
$QH^{\cdot}$	UHF	−41.5	−29.2	−74.6	−117.4	−75.4 (−74.5)	−0.6
	ROHF	−32.2	−17.7	−63.1	−105.2	−65.2 (−62.1)	7.6
$QH_2^{+\cdot}$	UHF	121.2	130.4	84	36.9	69.5	146.4
	ROHF	124	134.4	89.1	41.2	75.7	151.2

Values in parentheses for VIII, c are for route B (Scheme 3).

tive increasing order with respect to the two parameters:

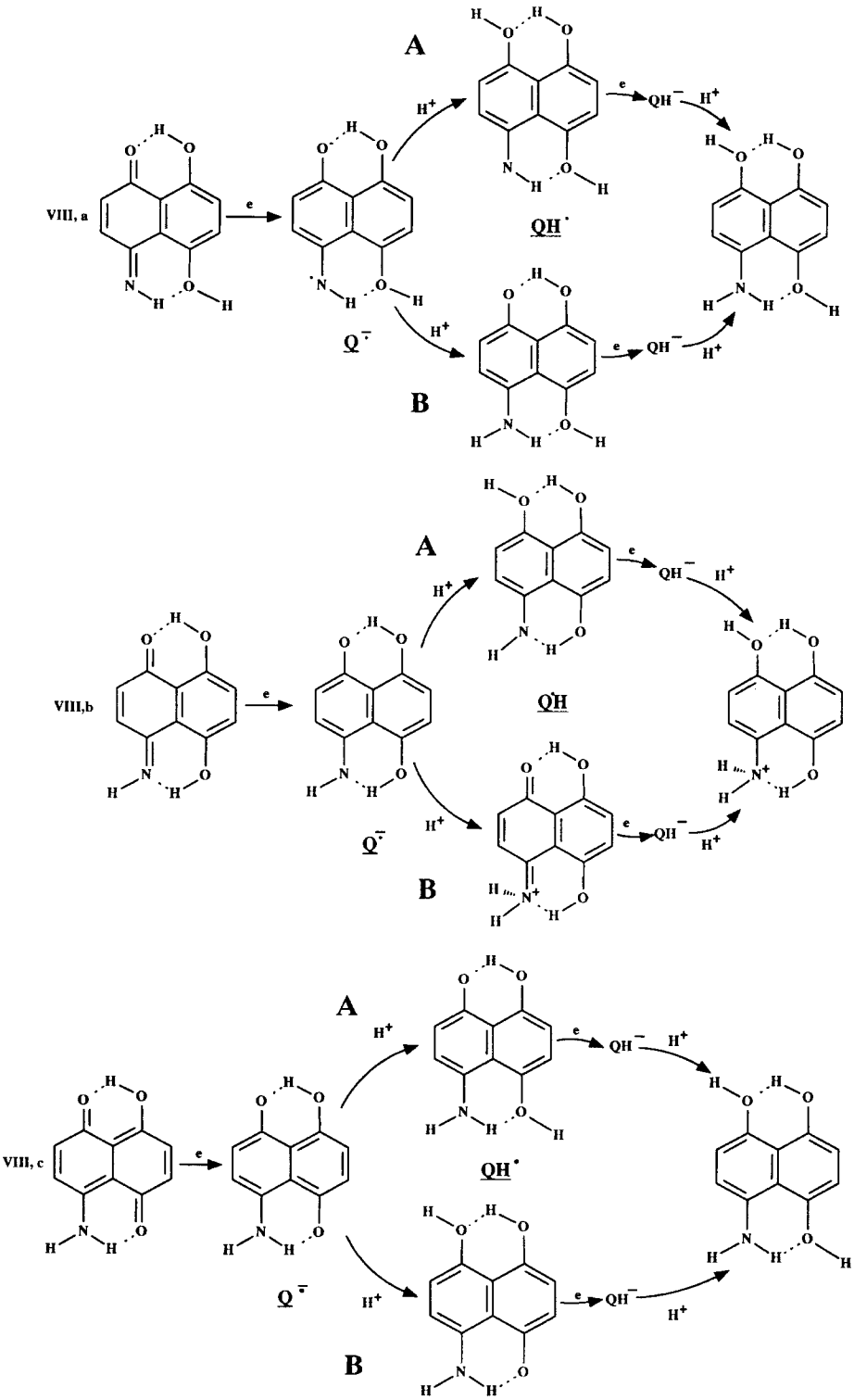
	IP_{ad}	EA_{ad}
Q	: VIIIc < VII < VI < V	V < VI < VIIIc < VII
$Q^{-\cdot}$	: V < VI < VIIIc < VII	V < VI < VII < VIIIc
$QH^{\cdot}$	: VIIIc < VII < VI < V	VIIIc < V < VI < VII
QH^{-}	: VIIIc < V < VI < VII	V < VIIIc < VI ~ VII
QH_2	: VIIIc < VII < VI < V	VIIIc < VI ~ VII < V

The difference in the IP_{ad}'s of the various redox states of VIIIc and VI are greater than 7 kcal/mol except for $Q^{-\cdot}$. It should, thus, be easier to oxidize redox states of VIIIc than it is to oxidize the corresponding redox states of VII. The EA_{ad}'s of VIIIc are also lower than that of VII, except for species $Q^{-\cdot}$. This would suggest that species from VIIIc should be more difficult to reduce compared to corresponding redox states of VII (except for $Q^{-\cdot}$). While the differences in the magnitudes of the IP_{ad}'s and EA_{ad}'s of VIIIc and VII are significant, they are not very large and the comparison can only be made in a relative sense. The data clearly suggest that the reducing capacity of VIIIc is not lower than that of VII. On the other hand, the oxidizing capacity of VIIIc seems to be somewhat diminished. Nevertheless, the relative diminished oxidizing capacity of VIIIc does not seem to be convincing to predict definitively a reduced cardiotoxicity for VIIIc. It is interesting to note here that both the reducing and oxidizing capacities of the redox states of VI are diminished relative to that of VII, except for the reducing capacities of species $Q^{\cdot -}$ and QH^{-}.

The redox states of 1,4-benzoquinone (IV), benzoquinone imine (IX), and V–VIII (Chart I) can also be compared using the electronegativity data presented in Tables I and II. For all cases, the decreasing order with respect to the magnitudes of the electronegativity value follows: $Q > QH^{\cdot} > QH_2 > Q^{\cdot -} > QH^{-}$. Since lower electronegativity values reflect a greater tendency to lose electrons, the reverse of the foregoing order gives the decreasing order in the tendency to lose electrons. Considering the absolute hardness values (Table I), a single order cannot be used for all the systems considered. The X data in Tables I and II can also be used to assess the effect of substituents. Comparing the electronegativity of each of the five redox states of the parent molecules (V–VIIIc), the following qualitative to semiquantitative relative decreasing order can be obtained:

	X_{ad}	X_V
Q	: V > VI > VII > VIIIc	(same as in X_{ad})
$Q^{-\cdot}$	: VIIIc > VII > VI > V	VII > VIIIc > VI > V
$QH^{\cdot}$	: VI > VII > V > VIIIc	(same as in X_{ad})
QH^{-}	: VII > VI > VIIIc > V	VII > VI > V > VIIIc
QH_2	: V > VI > VII > VIIIc	(same as in X_{ad})

Again, the reverse of these relative orders gives a decreasing order in the relative tendency to lose electrons by the respective species. Considering VII and VIIIc, for example, the absolute electronegativity of the different redox states of VIII is lower than that for the corresponding redox states of VII, except the X_{ad} of $Q^{-\cdot}$. This suggests that oxidation of the reductively activated forms of VIIIc should be easier than that of VII. The reactivity of redox states that can be obtained from different forms of VIII (Scheme 3, a–c) can also be examined using the data in Table I (entries for VIII a–c). Despite the variations in electronegativity values, none of the forms has values that will make it less reactive than VII if electronegativity considerations are used to predict the relative tendency of VII and VIII to lose electrons. On the other hand, at least for Q, $QH^{\cdot}$, and QH_2, the electronegativity of VI is higher



SCHEME 3. Possible routes for the reductive activation of 5,8-dihydroxy-1,4-naphthoquinone imine(VIII).

TABLE IVa.
Some Electron and Proton Attachment Reactions and Ionization of $QH^{\cdot}$ and QH_2.

1.	$Q + e \rightarrow Q^{\cdot -}$	6.	$QH^{\cdot} \rightarrow QH^{+} + e$
2.	$Q^{\cdot -} + H^{+} \rightarrow QH^{\cdot}$	7.	$QH_2 \rightarrow QH_2^{+\cdot} + e$
3.	$QH^{\cdot} + e \rightarrow QH^{-}$	8.	$Q + 2e \rightarrow Q^{-2}$
4.	$QH^{-} + H^{+} \rightarrow QH_2$	9.	$Q^{\cdot -} + e \rightarrow Q^{-2}$
5.	$Q^{-2} + H^{+} \rightarrow QH^{-}$		

than that of VII. This suggests that VI has a diminished capacity to lose electrons. In light of the proposed mechanism of action [vide supra Eqs. (3) and (4), Introduction] of the drugs, electron transfer from $QH^{\cdot}$ and QH_2 of VI to O_2 should be diminished (relative to that from $QH^{\cdot}$ and QH_2 of VII), implying a reduced toxicity for VI.

Enthalpies of electron and proton attachment reactions can be compared with the ionization of $QH^{\cdot}$ and QH_2 (Table IVa) using the data in Table IVb. The electron attachment reactions 1 and 3 in Table IVa are exothermic, while reactions 8 and 9 are not. Conversely, the proton attachment reactions are endothermic, except for reaction 5. The ionization enthalpies of $QH^{\cdot}$ and QH_2 (reactions 6 and 7) are sufficiently positive that energy released in reactions such as 1 and 3 cannot offset the positive enthalpy. The calculated enthalpies suggest that the energy given off in reactions 1, 3, and 5 may be sufficient to make reactions 2, 4, 8, and 9 feasible.

ENTHALPIES FOR SELF-EXCHANGE PROCESSES

Electron transfer reactions are possible between the various redox states, and Table Va shows some possible cases. The calculated enthalpies (for the gas phase forward reaction) for these self-exchange processes are given in Tables Vb and Vc. Considering the RHF/ROHF results in Table Vc, reaction 10 is exothermic and 12–15 are endothermic to strongly endothermic; reaction 11 may be a borderline case, while 16 is highly exothermic. In nonaqueous media and in aprotic solvents, of the listed reactions, reaction 16 is the only possible one since one or both reactants in the other reactions would require a proton source for their formation. Although the reverse of reaction 13 should be highly exothermic, the concentration of Q^{-2} may not build up in aqueous media and protic solvents, and hence the reaction is probably unlikely to occur to any significant degree. The reverse of reactions 14 and 15 should be exothermic, but QH^{+} and $QH_2^{+\cdot}$ must be produced by some other reaction. Such a reaction may not, however, be feasible.

Formation of QH^{+} and $QH_2^{+\cdot}$ via protonation of Q and $QH^{\cdot}$, respectively, or via ionization (Table IVa) should be endothermic and thus probably unlikely to occur; for example, the enthalpies of protonation of $QH^{\cdot}$ of VII and VIIIc are about, respectively, 146 and 141 kcal/mol; hence the reaction is not enthalpically favorable. Of the reactions listed, reactions 10 and 16 and possibly 11 are the most likely ones to occur. Consideration of the differences in the absolute electronegativities of the reactant redox states for each reaction shows that the differences are not as substantial for reactions 13–15, for which the differences are less that 1 eV. The data seem to suggest that for a negative reaction enthalpy, the difference in the electronegativities of the reactants that drive an electron transfer process should be significantly greater than

TABLE IVb.
RHF / ROHF-Calculated Reaction Enthalpies (kcal / mol) for Electron and Proton Attachments and Ionization Enthalpies of $QH^{\cdot}$ and QH_2.

Reaction	IV	V	VI	VII	VIII, c
1	−48.7	−45.3	−48.9	−52.1	−49.8
2	41.6	43.5	47.2	52.7	39.6 (42.7)
3	−54.4	−54.8	−60.6	−61.9	−48.6 (−53.6)
4	20.9	28.7	35.8	37.4	28.7 (30.5)
5	−87.7	−75.9	−71.8	−61.3	−54.9 (−56.8)
6	184.8	171.2	173.9	168.4	153.8 (160.1)
7	189.7	178.2	177	170.9	160.9
8	26.2	19.3	9.5	0	−3.9
9	74.9	64.6	58.4	52	45.9

Values for reactions 1–4 are the same as those for steps 1–4, respectively, in Table IIa, Section II.

TABLE IVc.
Adiabatic Ionization Potentials (IP_{ad}) and Electron Affinities (EA_{ad}) (kcal / mol).[a]

	IV	V	VI	VII	VIII, c	IX
	Adiabatic IP					
Q	244.4	223.1	208	197.7	177.4	233.1
$Q^{\cdot -}$	48.7	45.3	48.9	52.1	49.8	40.7
$QH^{\cdot}$	184.8	171.2	173.9	168.4	153.8 (160.1)	162.1
QH^-	54.4	54.8	60.6	61.9	48.6 (53.6)	49.1
QH_2	189.7	178.2	177	170.9	160.9	174.1
	Adiabatic EA					
Q	48.7	45.3	48.9	52.1	49.8	40.7
$Q^{\cdot -}$	−74.9	−64.6	−58.4	−52	−45.9	−80.5
$QH^{\cdot}$	54.4	54.8	60.6	61.9	48.6 (53.6)	49.1
QH^-	−113.7	−89.1	−81.2	−81	−82.7 (−84.8)	−155.5
QH_2	3.9	19.7	18.5	18.9	10.9	0.3

[a] H_f's used for radicals were calculated by the ROHF method. Values in parentheses for VIII, c are for route B (Scheme 3).

TABLE Va.
Some Possible Electron Exchange Reactions.[a]

10.	$QH^- + QH^{\cdot} \rightarrow QH_2 + Q^{\cdot -}$	14.	$QH_2 + QH^{\cdot} \rightarrow QH_2^{+\cdot} + QH^-$
11.	$QH^- + Q \rightarrow QH^{\cdot} + Q^{\cdot -}$	15.	$QH^{\cdot} + Q \rightarrow QH^+ + Q^{\cdot -}$
12.	$QH_2 + Q \rightarrow 2QH^{\cdot}$	16.	$Q + Q^{-2} \rightarrow 2Q^{\cdot -}$
13.	$Q^{\cdot -} + QH^- \rightarrow Q^{-2} + QH^{\cdot}$		

[a] Reaction 10 involves proton transfer, while reaction 12 involves electron transfer followed by proton transfer.

TABLE Vb.
RHF / UHF-Calculated Reaction Enthalpies (kcal / mol) for Electron Exchange Reactions.

Reaction	IV	V	VI	VII	VIII[b]
10	−14.5	−7.2	−4.3	−7.3	1.7
					−22.1
					−14.5
					−14.6
11	−6.7	−5.8	−4.1	−6.5	−10.8
					−16.6
					−11.3
					−14.1
12	7.8	1.4	0.1	0.7	−12.6
					5.4
					3.2
					0.5
13	123.1	111.7	111.8	106.0	109.5
					103.7
					106.8
					104.1
14	144.6	130.9	122.8	119.8	116.3
15	142.3	133.6	132.2	124.3	115.1
16	−127.6	−117.5	−115.9	−112.6	−120.2

[b] Values are for different forms of VIII (Scheme 3).

TABLE Vc.
RHF / ROHF-Calculated Reaction Enthalpies (kcal / mol) for Electron Exchange Reactions[c] 10 – 16.

	IV	V	VI	VII	VIII, c	IX
10	−20.7	−14.8	−11.4	−15.2	−11 (−12.2)	−6.1
11	5.7	9.5	11.7	9.9	−1.2 (3.8)	8.4
12	26.4	24.3	23.1	25.1	9.8 (16)	35.5
13	127.1	119.4	119	113.9	94.5 (99.5)	129.6
14	135.3	123	116.4	109	112.3 (107.3)	125
15	136.1	125.9	125	116	104 (110.3)	127.4
16	−123.6	−109.9	−107.3	−104	−95.7	−65.4

[c] Values in parentheses for VIII, c are for route B (Scheme 3).

1 (at least in the absence of influences of medium and field effects). In any case, consideration of the reaction enthalpies in Table Vc strongly suggests that none of the reactions considered are favorable in the case of VII and unfavorable in the case of VIIIc. The fact that the magnitudes of the reaction enthalpies are different in the two cases does not lead one to conclude that the reactivity of VIIIc is diminished enough to render VIIIc less toxic.

ENTHALPIES FOR ELECTRON TRANSFER TO O_2

Some possible electron transfer reactions are listed in Table VIa, and the corresponding gas phase reaction enthalpies are shown in Tables VIb and VIc. For each reaction, three sets of values are given in Table VIb; the first is for electron transfer to singlet $O_2(^1\Delta gO_2)$[‡] and the second to ground state $O_2(^3\Sigma g^- O_2)$. The difference in the two values reflects the difference in the stabilities of singlet and ground state O_2. The ground state O_2 is reported to be more stable than the singlet state by 22.4 kcal.[27] The stabilization calculated by PM3 (22.6 kcal) was in very good agreement with this stabilization, while AM1 gave a stabilization of 27.7 kcal (the ground state O_2 was calculated as a triplet in the UHF calculation). For this reason, PM3 values were used in Table VIb to compare the two states of O_2. The third entry for each reaction in Table VIb used ROHF-calculated H_f's for all the radicals in Table VIa.

The reaction enthalpies for reactions 17 and 19 (focusing on data in Table VIc) are substantially lower than those for 18 and 20, although both are still endothermic. Reactions 17–20 have to be, therefore, coupled with other exothermic reactions to be enthalpically favorable. On the other hand, reaction 21 is exothermic even for the less reactive ground state O_2 case. Comparing VII and VIIIc, reaction 21 is more favorable in the case of VII than in the case of VIIIc, and reactions 17–20 are more favorable in the case of VIIIc. Overall, the data suggest that the electron transfer capacity from the redox states of VIIIc to O_2 is not diminished when compared to that of VII.

Reactions 18 and 20, which are electron transfer reactions from $QH^{\cdot}$ and QH_2 to O_2, respectively, are not feasible even if they may be coupled to exothermic reactions since that will mean coupling to reactions that have reaction enthalpies on the order of 140 kcal/mol or greater. In light of this,

[‡] We acknowledge here the comments by a reviewer, who pointed out to us that the singlet states given by default by such programs as AMPAC and MOPAC are closed-shell states, which will be some mixture of the $^1\Sigma$ and $^1\Delta$ states, and that the AM1–PM3 difference for the O_2 singlet–triplet splitting may not have much significance.

TABLE VIa.
Some Possible Electron Transfer Reactions from Various Redox States to Molecular O_2.

17. $Q^{\cdot -} + O_2 \rightarrow Q + O_2^-$
18. $QH^{\cdot} + O_2 \rightarrow QH^+ + O_2^-$
19. $QH^- + O_2 \rightarrow QH^{\cdot} + O_2^-$
20. $QH_2 + O_2 \rightarrow QH_2^{+\cdot} + O_2^-$
21. $Q^{-2} + O_2 \rightarrow Q^{-\cdot} + O_2^-$

TABLE VIb.
Calculated Reaction Enthalpies (kcal / mol)[a] for Electron Transfer from Various States to Singlet and Ground State O_2.

Reaction	IV	V	VI	VII	VIII, a	VIII, c	IX
17	20.1	17.3	21.4	24.6	17.5		
	42.7	39.8	44.1	47.2	40.1		
	17	**13.6**	**17.2**	**20.3**		**18.1**	**9**
18	162.4	151	153.7	148.9	132.6		
	185	173.6	176.3	171.5	155.2		
	153	**139.5**	**142.2**	**136.7**		**122.1 (128.4)**	**136.4**
19	13.4	11.6	17.4	18	6.7		
	36	34.2	40	40.6	29.3		
	22.7	**23.1**	**28.9**	**30.2**		**16.9 (21.9)**	**17.4**
20	155.2	142.5	140.5	137.9	123		
	180.6	165.1	162.8	160.5	145.6		
	158	**146.5**	**145.2**	**139.2**		**129**	**142.4**
21	−109.7	−100.1	−94.9	−88	−102.7		
	−87.1	−77.5	−72.3	−65.4	−80.1		
	−106.6	**−96.3**	**−90.1**	**−83.7**		**−77.6**	**−112.2**

[a] The first and second entries for each reaction are for singlet ($^1\Delta g$ O_2) and ground ($^3\Sigma g^-$ O_2) state O_2, respectively. The third entry (boldface) for each reaction used ROHF-calculated H_f's for radicals and the singlet O_2 case. H_f's used for O_2 were 18.4 and −4.2 kcal / mol for singlet and ground state O_2, respectively (calculated with PM3) and −13.3 kcal / mol for O_2^- (PM3 calculated); the UHF and ROHF H_f's were the same for O_2^-.

two other reactions may be considered:

22. $OH^{\bullet} + O_2 \rightarrow Q + HO_2^{\bullet}$ [vide supra Eq. (4)]

23. $QH_2 + O_2 \rightarrow QH^{\bullet} + HO_2^{\bullet}$ [vide supra Eq. (3)]

The reaction enthalpies for 22 and 23 can be shown to be substantially lower. For example, for Q = VII, the reaction enthalpies are −12.5 and 12.6 kcal/mol, respectively, for singlet O_2. The corresponding values for Q = VIIIc are −1.7 (−4.8 for route B, Scheme 3) and 8.1 (11.2 for route B, Scheme 3). This suggests that reaction 22 is more favorable than reaction 23. Reaction enthalpies for the other systems can be calculated using the data in Tables I and IIIb and the heat of formation for $HO_2^{\bullet}$ (= −11.2 kcal/mol, AM1 ROHF calculated). It is clear then that reactions 22 and 23 are more feasible than reactions 17–20. With proton transfer from QH^+ and $QH_2^{+\bullet}$ to O_2^- (in reactions 18 and 20, respectively), reactions 18 and 20 lead to 22 and 23, respectively.

Discussion

The quantum chemical calculations performed on the model systems do not support the rationalization that 5IDN is difficult to reduce and/or oxidize.[5,6] Absent some metabolic pathway that is unlike that for the anthracycline class of drugs (e.g., daunomycin) 5IDN should be just about as capable to reduce molecular oxygen. Since it had

TABLE VIc.
RHF / ROHF AM1-Calculated Reaction Enthalpies (kcal / mol)[b] for Electron Transfer from Various States to Singlet O_2.

Reaction	IV	V	VI	VII	VIII, c	IX
17	25.3	21.9	25.5	28.6	26.4	17.3
18	161.4	147.8	150	145	130.4 (136.7)	144.7
19	31	31.4	37.2	38.5	25.2 (30.2)	25.7
20	166.3	154.8	153.6	147.5	137	150.7
21	−98.3	−88	−81.8	−75.4	−69.3	−103.9

[b] H_f's for O_2 and O_2^- were 0.7 and −22.7 kcal / mol, respectively. UHF and ROHF results were the same for O_2^-.

TABLE VII.
Some Energetic Parameters for VI and for Keto and Enol Forms of the Hydroquinones of VI and VIII. (Charts IIIa and IIIb).

	H_f	HOMO	LUMO	X_V	N_V
1a	−61.4	−9.619	−1.678	5.649	3.971
b	−56.1	−9.625	−1.483	5.554	4.071
c	−37.6	−10.461	−1.98	6.221	4.241
2a	−87	−8.291	−0.477	4.384	3.907
b	−85.8	−8.019	−0.206	4.113	3.907
c	−88.6	−8.042	−0.336	4.189	3.853
d	−87.4	−8.072	−0.458	4.265	3.757
e	−87.9	−8.153	−0.431	4.292	3.861
f	−85.2	−8.054	−0.317	4.186	3.819
3a	−78.7	−8.865	−0.554	4.71	4.156
b	−84.8	−8.815	−0.767	4.791	4.024
c	−83.9	−8.852	−0.838	4.845	4.007
d	−78.1	−8.875	−0.616	4.746	4.13
4a	−85.17	−7.461	−0.117	3.789	3.672
b	−83.41	−7.666	−0.283	3.975	3.692
c	−86.89	−7.89	−0.412	4.151	3.729
d	−45.48	−7.264	−0.84	4.052	3.212
e	−53.97	−6.83	−0.396	3.613	3.217
f	−75.96	−8.733	−0.89	4.812	3.922

H_f (heat of formation) is in kcal / mol; all other parameters are in eV.

been suggested that the inefficiency of 5IDN to catalyze the reduction of molecular oxygen is due to the facile formation of I (from 5IDN) whose hydroquinone, II, tautomerizes to III (Scheme 1),[7] we investigated the effects of keto–enol transformation and hydrogen bonding on chemical reactivity in model system VI. Some AM1 calculated energetic parameters are tabulated (Table VII) for the structures in Chart III. Hydrogen bonding stabilizes structure 1a by 5.3 kcal/mol (compared to 1b), but the difference in their absolute electronegativity is only 0.095 eV. The effect of hydrogen bonding on the stabilization when comparing structures 1a–c, 2a–f, and 3a–d can easily be gleaned from the data. This stabilization can be up to 3.4 kcal/mol in the enol forms, while it can be as much as 6.7 kcal/mol in the keto forms. Comparing the electronegativities of keto and enol forms, the electronegativities of the keto forms are larger by 0.326 to 0.732 eV. The difference in heats of formation between the keto and enol forms is not substantial, and a significant population of the keto form may not be present at ordinary temperatures. Based on the solvation energy data[24] presented in Table VIII, the keto forms are slightly better solvated, particularly 3a and 3d. The magnitudes of the differences in the solvation energies do not, however, suggest that the population of the keto forms can be much more favored in solution than in the gas phase. Since an increase in electronegativity leads to a decrease in the tendency to lose electrons, the keto forms should be considerably less reactive in electron transfer reactions. The electronegativities of the keto forms of the reduced states (3a–d) when compared to that of O_2 (6.2 eV)[28] are, however, still low enough that a redox couple reaction should be feasible. The AM1- and PM3-calculated X_V values for O_2 were 5.51 and 5.86 eV, respectively. These values are higher by about 0.8 eV when compared to those for 3a–d, although they are still higher (by > 1.2 eV) than those for enol forms 2a–f and 4a–c. Consideration of electronegativity values is not therefore thought to be sufficient to explain the reduced reactivity of the keto forms of 5IDN metabolites. In this regard, other functions, such as molecular charge and medium effects, can attenuate the driving force due to electronegativity difference alone.[12] This argument is clearly supported by the data in Table IX, which provides the coefficients of the HOMO molecular orbitals of selected systems. As reflected by the values of the coefficients for the P_Z atomic orbitals on atoms 1–5, the electron densities on the keto moiety of structures

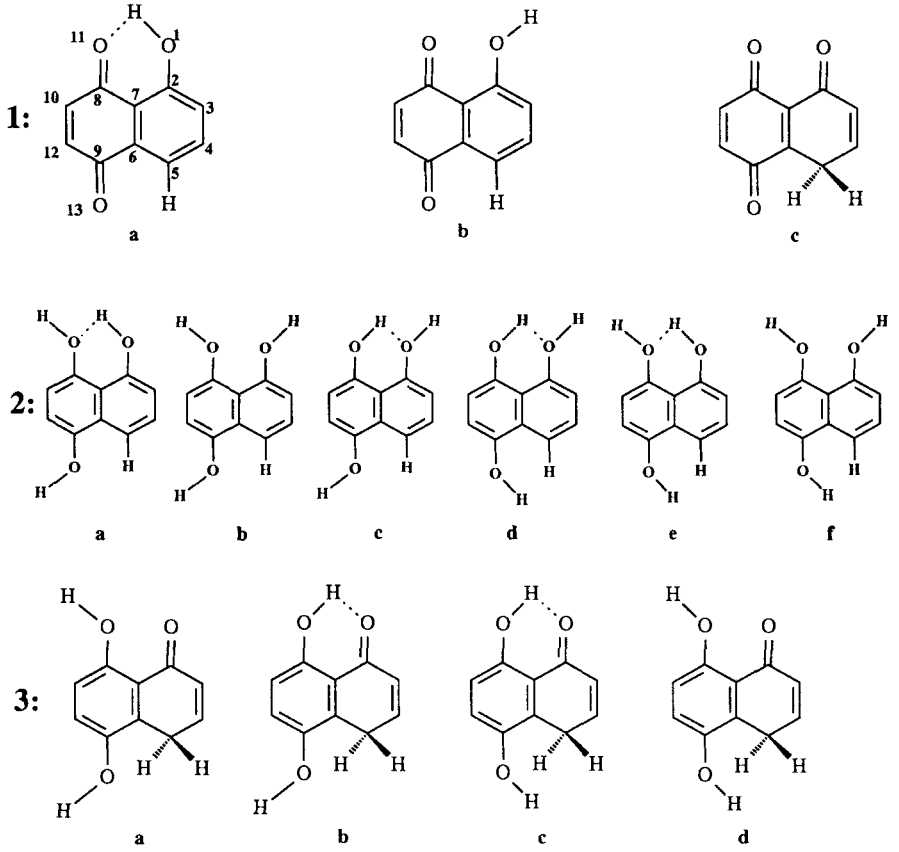


CHART IIIa. Various structures of 5-hydroxy-1,4-naphthoquinone (juglone, VI) and enol / keto forms of its hydroquinone.

3a and 3b are so low that the keto moiety probability does not contribute to the electron exchange process. Compared to structures 2a and 2b, structures 3a and 3b should be less efficient.

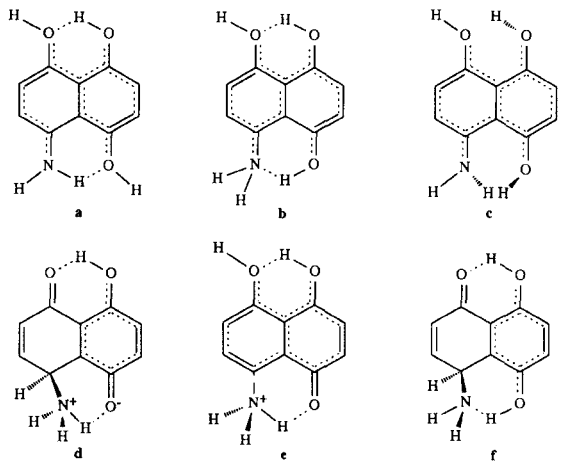


CHART IIIb. Various structures for the hydroquinone of 5,8-dihydroxy-1,4-naphthoquinone imine (VIII).

GEOMETRICAL CONSTRAINTS

The geometries of 3a–d are not planar. This nonplanarity places a restriction on the direction approach reactants must take to approach each other in order for electron transfer to occur. This geometrical requirement places a severe limitation, particularly more so on the reactions in Table Va than those in Table VIa, because in the latter the O_2 molecule should have a better chance of closest approach for maximum overlap due to its smaller size. The enol-to-keto form conversion can thus be expected to affect the reactions in Table Va more dramatically. Consequently, it was also of interest to consider the keto forms of the hydroquinone of VIII (Chart IIIb). Some AM1 calculated parameters for 4a–f are also included in Table VII. (Not all rotamers and/or tautomers of the hydroquinone of VIII are included here.) As the data indicate, there are substantial differences in the heats of formation. Of the keto forms, 4d and 4e are particularly unlikely to form. On the other hand, consideration of the solvation energies for 4a–e clearly

TABLE VIII.
AM1 – SM2-Calculated Solvation Free Energies of Various Keto and Enol Forms Shown in Charts IIIa and IIIb.

Structure	G_{ENP}	G_P	G_{CDS}	G_{P-CDS}	G_S
1a	−65.75	−5.59	−5.1	−10.59	−70.85
b	−61.58	−7.2	−4.88	−12.08	−66.47
c	−43.98	−9.91	−2.99	−12.89	−46.7
2a	−91.23	−5.14	−9.35	−14.49	−100.58
b	−90.36	−5.63	−9.04	−14.67	−99.39
c	−92.84	−5.28	−9.35	−14.62	−102.19
d	−91.62	−5.17	−9.4	−14.56	−101.01
e	−92.05	5.12	−9.39	−14.51	−101.44
f	−89.02	−5.49	−9.06	−14.55	−98.69
3a	−85.17	−9.33	−7.21	−16.54	−92.38
b	−90.07	−7.52	−7.37	−14.89	−97.43
c	−89.14	−7.36	−7.44	−14.8	−96.57
d	−84.63	−9.22	−7.25	−16.46	−91.88
4a	−90.27	−6.61	−11.92	−18.53	−102.2
b	−88.01	−5.72	−12.16	−17.89	−100.17
c	−91.14	−5.16	−11.85	−17.01	−102.99
d	−70.39	−34.51	−7.65	−42.16	−78.04
e	−75.42	−29.77	−9.97	−39.74	−85.4
f	−82.91	−9.33	−10.13	−19.45	−93.04

G_P = solution polarization energy; G_{CDS} = cavity-dispersion-solvent structure free energy; $G_{P-CDS} = G_P + G_{CDS}$; $G_{ENP} = G_{EN} + G_P$; G_{EN} = sum of solute electronic kinetic and electronic–nuclear coulombic energies; $G_S = G_{EN} + G_{P-CDS}$.

TABLE IX.
Coefficients of Highest Occupied Molecular Orbitals (HOMO) of selected systems 1 – 3(a, b).[a,b]

	1a	1b	2a	2b	3a	3b
	−9.619	−9.625	−8.291	−8.019	−8.865	−8.815
Atom No.[c]						
1	0.39	0.39	−0.26	0.2	0.06	−0.06
2	−0.43	−0.44	0.36	−0.3	−0.01	0.02
3	−0.36	−0.32	0.35	−0.28	0.08	−0.09
4	0.12	0.21	−0.19	0.22	0.05	−0.05
5	0.51	0.51	−0.42	0.36	−0.08	0.05
6	0.29	0.22	−0.04	−0.06	0.3	−0.35
7	0.39	−0.4	0.05	−0.003	−0.23	0.23
8	−0.03	−0.01	−0.32	0.37	−0.42	0.41
9	0.01	0.01	0.38	−0.4	0.48	−0.48
10	0.005	0.01	−0.30	0.35	−0.34	0.36
11	0.15	0.16	0.19	−0.24	0.33	−0.34
12	0.02	0.01	0.25	−0.29	0.24	−0.18
13	−0.13	−0.09	−0.08	0.24	−0.32	0.32

[a] See Chart III.
[b] The first row provides the HOMO energies in eV.
[c] The numbering is as shown in Chart III (1a).

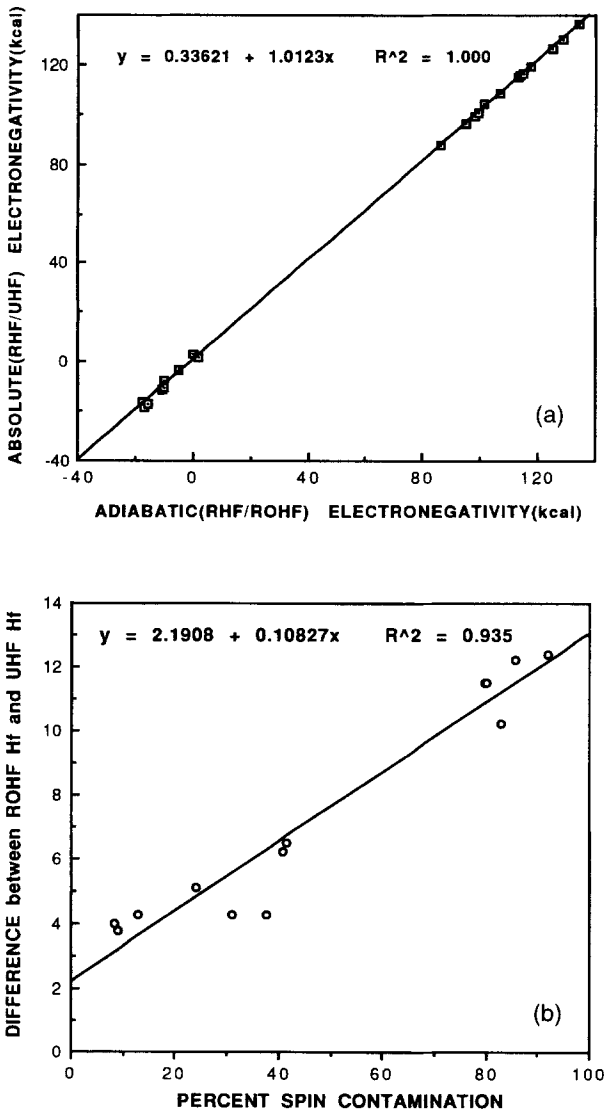


FIGURE 1. (a) Plot of absolute (RHF / UHF) electronegativity vs. adiabatic (RHF / ROHF) electronegativity (kcal). (b) Plot of the difference between ROHF H_f and UHF H_f ($\Delta\Delta H_f$, for radicals, kcal / mol) vs. percent spin contamination.

indicates that the G_P terms for 4d and 4e are four to five times greater than those for 4a and 4b. However, 4d and 4e, if present, have a markedly different chemical hardness and hence should be more reactive. Interestingly, 4c has the largest electronegativity and hence should be a better electron acceptor in an electron exchange reaction, except that geometrical constraints due to its nonplanar geometry may play a significant role.

ELECTRONEGATIVITY VERSUS OTHER FACTORS

An attempt has been made in this work to assess if calculated absolute electronegativity parameters can enable the prediction of the relative chemical reactivities of structurally closely related systems. The findings suggest that the parameter should be adequate to compare the reactivity of different redox states and to get some general trends with regard to substituent effects. Based on the calculated results, the =NH substitution for =O (VIII vs. VII) should not change the redox capacity to the extent that VIII (and its redox states), when part of a molecule, as in 5IDN, should lead to no (or even less) cardiotoxicity. However, the incorporation of absolute electronegativity (and absolute hardness) parameters into molecular orbital theory by considering only HOMO and LUMO energies may not always be adequate. For some species, particularly for redox states such as $Q^{\bullet -}$ and Q^{-2} considered here, the HOMO and the second HOMO may have energies that are fairly close and the second HOMO may actually participate in chemical reactions. Thus, interpretation of chemical reactivity in terms of frontier orbitals only may not be adequate for all the redox states of the model systems investigated here.

EFFECT OF *N* SUBSTITUTION

In the search for new antineoplastic drugs, amido-anthraquinone derivatives hoped to be devoid of severe toxicity have been investigated.[29] The results showed that at least most of the derivatives were effective in inhibiting DNA synthesis, cell growth, and DNA damage, results that were correlated with binding affinity.[29] There was, however, no report on the toxicity of the drugs, although reduction in toxicity is always one of the goals of the search for new anthraquinone drugs. Assuming that the toxicity of these drugs is probably intimately tied to orbitally controlled reactivity, the calculations presented here provide some insight into the toxicity of the amido derivatives. *N*-substituted analogs should lead to reduced electron affinity and reduced electronegativity (or increased electronic chemical potential). The lowering in electron affinity should result in reduced competition with O_2 for an electron in processes such as the electron transport chain of a mitochondrion. This is a desirable effect. On the other hand, $O_2/ Q^{-\bullet}$ (VIII) and O_2/QH^- (VIII) should be bet-

ter redox couples, meaning that electron transfer to O_2 is more feasible from reduced forms of VIII.

The mode of action of the anthracyclines and their derivatives is still elusive because most experimental evidence dealt with the correlation of activity to the mode of binding. Reductive activation of the drugs, however, causes considerable reorganization in electronic structure, which manifests as changes in geometry, electron affinity, and electronegativity (or electronic chemical potential), thereby altering the capability for intra- and intermolecular electron transfer reactions involving short-lived species and O_2. A case can thus be made that computational approaches can give more insight into the elusive mode of actions of the anthracyclines.

THERMODYNAMIC VERSUS KINETIC CONTROL

Thermodynamic calculations such as presented here on reaction enthalpies do not provide information on rates of reactions, and it may be that the kinetics (the rate and/or mechanism) of the reactions govern the reactivity of the anthracyclines. From a mechanistic point of view, several considerations can be made. It has been reported that semiquinones can form stable complexes with parent quinones with equilibrium constants ranging from 400 to 15,000 M^{-1}. The complex thus formed then reacts with O_2 to produce $O_2^{-\cdot}$ (reactions 24 and 25).[30]

$$24.\quad Q^{-\cdot} + Q \rightarrow Q_2^{-\cdot}$$

$$25.\quad Q_2^{-\cdot} + O_2 \rightarrow 2Q + O_2^{-\cdot}$$

The efficacy of reaction 25 will depend on the complexation reaction (24). If reaction 24 is an important step in the overall formation of $O_2^{\cdot -}$, the role played by the keto and enol forms of the reactants can be primarily a direct consequence of the geometrical constraints on the efficacy of complexation. Since the keto forms cannot be expected to lead to optimum complexation, reaction 24, and consequently reaction 25, can be severely affected if keto forms are the reactants. Thus, if the role of reaction 24 in the redox-cycling reactions of the anthracycline leading to formation of O_2^- can be established, the role played by the keto and enol forms of the metabolites can be put on a firmer ground. The reduction of O_2 by an electron donor, D, can also take place via two mechanisms[31]:

$$26.\quad D + O_2 \rightarrow D^{+\cdot} + O_2^{-\cdot}$$

$$27.\quad D + O_2 \rightarrow D^+O_2^-$$

Reactions 26 and 27 are outer and inner sphere mechanisms, respectively. For reaction 26 to be efficient, as written, the reduction potential of D must be smaller than that of O_2. The alternative mechanism, reaction 27, can be feasible if a stable dioxygen adduct is formed (especially if D is a metal cation or a metal complex). Reaction 26 can be a feasible mechanism if it is followed by an irreversible reaction of D^+ with some other compounds. Reactions 18 and 20 are examples of reaction 26, and simple deprotonation of QH^+ and $QH_2^{\cdot +}$ in water can shift the endothermic reactions (18 and 20) to the right. The solvent or the reaction medium can play a major role in shifting reactions 26 and 27 to the right or left.

Reactive oxygen species such as $HO_2^{\cdot}$ and $QH^{\cdot}$ have been implicated as the species that are ultimately responsible for cardiotoxicity (and also anticancer activity).[31] To form $HO_2^{\cdot}$, O_2^- will have to abstract H^+ from solvent or from some other species, and the metabolites of the drugs can serve as such H^+ sources. But the metabolites should exist as enol forms to be effective H^+ donors. If they exist in the keto form, the formation of $HO_2^{\cdot}$ will not be facilitated, and this may be another way in which the keto form will affect the biological activity of the drugs and/or their metabolites.

POSSIBLE ROLE FOR Q^{-2}

Closer examination of the reaction enthalpies of reactions 1–21 reveals that reactions 5, 16, and 21 result in considerably more negative reaction enthalpies. It is also apparent that all three reactions involve Q^{-2}, which is the two-electron reduction product of Q. In aprotic media and/or a hydrophobic environment, the reductive activation results in $Q^{-\cdot}$ and Q^{-2}, and not in $QH^{\cdot}$ and QH_2. Electron transfer from Q^{-2} to O_2 results in $Q^{-\cdot}$, which can, in turn, participate in reaction 17. Although reaction 17 is enthalpically unfavorable, coupling of reactions 21 and 17 leads to a net reaction:

$$Q^{-2} + 2O_2 \rightarrow Q + 2O_2^{-\cdot}$$

$$H = -46.8 \text{ kcal/mol (for VII)}$$

Reaction 17 can also be coupled with reaction 16 with a net reaction:

$$Q + Q^{-2} + 2O_2 \rightarrow 2Q + 2O_2^{-\bullet}$$
$$\Delta H = -46.8 \text{ kcal/mol (for VII)}$$

Similarly, in protic media, reaction 5 can be coupled with reaction 19, giving a net reaction:

$$Q^{-2} + H^+ + O_2 \rightarrow QH^{\bullet} + O_2^{-\bullet}$$
$$\Delta H = -22.8 \text{ kcal/mol (for VII)}$$

Similar calculations can be done for the foregoing coupled reactions for the other model systems, and the net reaction enthalpy turns out to be favorable. In light of these observations, it appears that once Q^{-2} is formed, the electron transfer process to O_2 is thermodynamically (enthalpically) favorable. Q^{-2} can be formed via a two-step process (reactions 1 and 9) or via a direct two-electron reduction (reaction 8). Reaction 8 is, of course, the net reaction from coupling 1 and 9. Interestingly, reaction 8 is more favorable for VIIIc and less favorable for VI when compared to VII. Moreover, the reaction enthalpies of the net reactions discussed earlier are either more favorable or as favorable for VIIIc when compared to VII. In summary, the analysis suggests several conclusions: (1) The electron transfer capacity of VIIIc and its various redox states, as well as its capacity for a two-electron reductive activation, is not diminished (compared to VII); (2) However, it appears that the reactivity of VI is diminished relative to that of VII; (3) Q^{-2} appears to play a key role in electron transfer processes to O_2, particularly in aprotic and hydrophobic media as opposed to $QH^{\bullet}$ and QH_2, which have been postulated to be responsible for the production of reactive dioxygen species (i.e., $O_2^{-\bullet}$). Hence, Q^{-2} may play a key role in cardiotoxicity.

Conclusion

The semiempirical MO calculations undertaken shed some light on the chemical reactivity of model systems for the anthracyclines. Adiabatic IPs and EAs, absolute or adiabatic electronegativity parameters, reaction enthalpy data for reductive activation, electron self-exchange, and electron transfer calculated for the model system VIII do not support that 5IDN should be redox incapacitated when compared to DN. However, the redox capacity of VI, which can be a model for aclacinomycin A and III, appears to be diminished. The results obtained on this system present a rationale for the reduced toxicities of aclacinomycin A and 5IDN. Moreover, keto–enol transformation and hydrogen bonding may play a significant role, not because they change the electronegativity dramatically, but because the electron configuration and/electron density and the geometry, such as planarity of the molecules, change as the result of the enol–keto transformation.

Some of the computational results are also instructive since they strongly suggest that, perhaps for the first time, the two-electron reduction product of the drugs, Q^{-2}, can play a key role in electron transfer processes to O_2, particularly in aprotic and hydrophobic media, as opposed to $QH^{\bullet}$ and QH_2, which have been postulated to be responsible for the production of reactive dioxygen species, such as $HO_2^{\bullet}$ and O_2^{-}. The results are thus highly suggestive that Q^{-2} may play a key role in cardiotoxicity. Further experimental investigation of this issue may be warranted.

Acknowledgments

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